Aquaculture 485 (2018) 154–159

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Short communication

Periodic bacterial control with peracetic acid in a recirculating aquaculture system and its long-term beneficial effect on fish health

Dibo Liu[a,b,*], David L. Straus[c], Lars-Flemming Pedersen[d], Thomas Meinelt[b]

[a] Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-University of Berlin, Invalidenstraße 42, 10115 Berlin, Germany

[b] Department of Ecophysiology and Aquaculture, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany

[c] U.S. Department of Agriculture, Agricultural Research Service, Harry K. Dupree—Stuttgart National Aquaculture Research Center, P.O. Box 1050, Stuttgart, AR 72160, USA

[d] Technical University of Denmark, DTU-Aqua, National Institute of Aquatic Sciences, Section for Aquaculture, North Sea Research Centre, P.O. Box 101, DK-9850 Hirtshals, Denmark

ARTICLE INFO

Keywords:
Water quality
Respiratory burst
Water disinfection
Gill histology

ABSTRACT

Fish in a recirculating aquaculture system (RAS) live with abundant microorganisms. These can become a health threat when the fish immune system cannot counterbalance the pathogenic microbial colonization. Therefore, microbial control in a RAS can potentially reduce the risk of infections and hence improve fish health. In the present study, a periodic microbial control was performed in a RAS with 16 tanks stocked with mirror carp (*Cyprinus carpio*) for 3 months. Half of the fish culture tanks were treated with 1 mg L^{-1} peracetic acid (PAA) twice per week, while the other half remained untreated. The water circulation was interrupted immediately before each PAA-treatment, and resumed after 3 h. The total aerobic bacterial density was similar in all culture tanks, except during the PAA-treatments and the concurrent circulation interruptions. During these periods, the bacterial density decreased up to 90% in PAA-treated water, while a 6-fold bacterial increase was observed in untreated water. In the first 2 months of treatment, PAA-exposed fish showed lower plasma cortisol concentration than the unexposed fish. Subsequently, the trunk kidney leukocytes of PAA-exposed fish showed stronger respiratory burst than the unexposed fish. By the end of the experiment, the PAA-exposed fish had better gill morphology, compared to the unexposed fish. The present study indicates that periodic disinfection of culture water in a RAS with PAA could transiently reduce the suspended bacteria density, modulate the fish stress response, and have an overall beneficial effect on fish health in the long term.

1. Introduction

Recirculating aquaculture system (RAS) technology is increasingly recognized as a sustainable option for modern, intensive aquaculture. The primary advantages are low water consumption, low environmental impact and high productivity (Martins et al., 2010). However, fish in RASs are typically raised at a high density with a long water retention time and a high feeding rate. This results in an accumulation of organic matter and micro-particles, which create a favorable condition for bacterial growth (Leonard et al., 2000; Blancheton et al., 2013; Rurangwa and Verdegem, 2015; Pedersen et al., 2017). These bacteria attach and colonize on all possible surfaces, including the mucosal surfaces of fish, namely the gill, the skin and the gut (Perez et al., 2010; Sunyer, 2013; Xu et al., 2013). The immune system at the fish mucosal surfaces can distinguish between the commensal and pathogenic bacteria. The former are tolerated, while the latter are counteracted by the up-regulated local humoral/cellular immune responses (Gomez et al., 2013). Under ideal conditions, the counteraction between pathogenic bacteria and the fish immune system would culminate in equilibrium without infections. However, in realistic RAS conditions, diseases associated with microbial proliferation or stress in fish have been reported (Noble and Summerfelt, 1996). Therefore, a general bacterial control could assist the fish immune system and reduce the risk of infections in RASs.

Several antibacterial techniques have been applied to RASs as prophylactic barriers. Ozone is an effective strategy, but ozone dosing is difficult and residual ozone or harmful disinfection byproducts can be acutely lethal to fish at concentrations as low as 0.01 mg L^{-1} (Summerfelt and Hochheimer, 1997), and chronic exposure to degradation residues of ozone can cause irreversible gill damage (Reiser

* Corresponding author at: Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences, Faculty of Life Sciences, Humboldt-University of Berlin, Invalidenstraße 42, 10115 Berlin, Germany.
E-mail address: liu@igb-berlin.de (D. Liu).

https://doi.org/10.1016/j.aquaculture.2017.11.050
Received 1 June 2017; Received in revised form 23 November 2017; Accepted 27 November 2017
Available online 28 November 2017
0044-8486/

et al., 2011). Ultraviolet (UV) irradiation is effective at inhibiting suspended bacteria in a RAS, but its efficiency is reduced with increasing turbidity (Gullian et al., 2012) and particulate matter content (Sharrer et al., 2005). Combination of UV with ozone was found to achieve stronger bacterial reduction effects (Sharrer and Summerfelt, 2007). Hydrogen peroxide (H_2O_2) is an oxidizing disinfectant and it degrades to oxygen and water quickly. The application of H_2O_2 in a RAS is limited by the relatively high recommended dose (≥ 15 mg L^{-1}) and the relatively low safe concentration to the biofilter (≤ 5 mg L^{-1}), leaving a fairly narrow therapeutic window (Pedersen and Pedersen, 2012).

Peracetic acid (PAA) is a stronger oxidizing disinfectant than H_2O_2, and degrades to biodegradable residues (Kitis, 2004). A nominal concentration of 1 mg L^{-1} PAA has been proven to reduce the *in vitro* growth of pathogenic bacteria in aquaculture, such as *Aeromonas salmonicida*, *Flavobacterium columnare* and *Yersinia ruckeri* (Marchand et al., 2012; Meinelt et al., 2015). Toxicology studies found that the 24-h no observed effect concentration was 1.3 mgL^{-1} PAA to channel catfish fry (Straus et al., 2012), and ranged from 1.9 to 5.8 mg L^{-1} PAA to juveniles of black fathead minnow, black-nose crappie, blue gill, blue tilapia, channel catfish, golden shiner, goldfish, grass carp, large-mouth bass, rainbow trout, sunshine bass and walleye (Straus et al., 2017). Furthermore, 1 mg L^{-1} PAA had no effect on ammonium removal of a RAS biofilter (Pedersen et al., 2009). Therefore, PAA has excellent potential to be applied at low concentrations in a RAS without adverse impacts on the fish and the biofilter, if added correctly (Liu et al., 2017b).

Treating the culture water with PAA in the presence of fish has multifaceted impacts on fish and bacteria, which may not necessarily result in a beneficial scenario. The aim of the present study was to determine if periodic bacterial control with PAA in a RAS could have a beneficial effect on the health status of the fish. It was hypothesized that regular applications of PAA could reduce the bacterial density in the culture water, and hence inhibit potential bacterial infection on fish. The hypothesis continues that despite the PAA-induced stress (Liu et al., 2017a), the immune system would benefit from PAA-treatments and result in a better health status in long term.

2. Materials and methods

2.1. Experimental design and treatments

An indoor research RAS, consisting of 16 culture tanks, a mechanical filter and a biofilter, was cleaned and disinfected prior to the experiment. The total water volume of the RAS was about 9.8 m^3. The flow rate of the system was 900 L h^{-1}, and the daily make-up water was 300 L. The culture tanks were identical rectangular tanks with a water volume of 300 L each and each tank was continuously aerated with an airstone.

Mirror carp (*Cyprinus carpio*) weighing 649 ± 183 g and of mixed sex were locally purchased and quarantined for three months. The fish were then moved to the RAS with a reconditioned, functioning biofilter. Each culture tank was stocked with 6 carp. The carp were acclimated for 6 months and fed daily at approximately 1% biomass commercial pellets until the study began. The temperature, pH and dissolved oxygen (DO) of the water was monitored daily and maintained stable (Temp = 21–22 °C, pH = 7.3–7.7, DO = 8–8.5 mg L^{-1}).

There were two treatments: a positive control group (8 tanks) and a PAA-treated group (8 tanks). Every Monday and Thursday, the water circulation was interrupted immediately before PAA-treatments, and restarted after 3 h. Tanks of the PAA-treated group received 750 µL Wofasteril® E400 (Kesla Pharma Wolfen GmbH, Greppin, Germany), resulting in a nominal PAA concentration of 1 mg L^{-1}. This approach was considered as a regular prophylactic water treatment (Rintamaki-Kinnunen et al., 2005; Pedersen et al., 2013). The positive control group received a sham treatment of 750 µL sterile distilled water. No PAA residues were detectable in the inlet water of any of the culture tanks, validated by the DPD-photometric method (Liu et al., 2014) after restarting the water circulation. Feeding was withheld 1 d prior to and during each treatment.

2.2. Sampling

Fish from two tanks (n = 12) were sampled from each group after the initial treatment and on a monthly basis thereafter for 3 months. At each sampling period, blood (n = 12) was collected from the caudal vein with heparinized syringes within 5 min after netting. Plasma (n = 12) was obtained after centrifugation at 13000*g* for 5 min and stored at − 20 °C until assayed. Subsequently, 6 random fish from each treatment group were euthanized. The head and trunk kidneys were aseptically removed, pressed through 70-µm EASYstrainer™ sterile mesh units (Greiner Bio-One International, Kremsmünster, Austria) and suspended in ice-cold RPMI-1640 wash medium (composed of phenol red + 10% distilled water + 100 U mL^{-1} Penicillin-streptomycin + 2 mM L-Glutamine + 25 mM Hepes buffer + 10 U mL^{-1} Heparin, 0.22 µm sterile filtered; Biowest, Nuaillé, France). At the final (3-month) sampling period, the second gill arch on the right side of each fish (n = 6) was removed, placed in separate cassettes and immediately fixed in Bouin's solution for histological analysis.

During two random PAA-treatments, when fish sampling was not scheduled, water samples were collected from the positive control and the PAA-treated culture tanks prior to and after the 3-h interruption. The colony forming units (CFUs) of total aerobic bacteria in water samples were determined in triplicate on agar plates using the drop plate method described by Meinelt et al. (2015).

2.3. Measurements of plasma cortisol

Plasma cortisol was determined using a Cortisol ELISA test kit (IBL International, Hamburg, Germany) according to the manufacture's instruction.

2.4. Histology

After the gill histology samples were fixed in Bouin's solution for 24 h, the solution was discarded and replaced with 70% ethanol, which was refreshed daily for the next 3 d. Gill samples were then dehydrated in a Shandon™ Excelsior™ Tissue Processor (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and they were manually embedded in paraffin blocks. Gill samples were decalcified for 5–8 h until sectioned with a rotary microtome to 3.5 µm thickness. Serial sections were transferred to a water bath (45 °C) and then placed on slides to be dried on a heating plate (45 °C). Finally, all slides were stained with hematoxylin and eosin. Cover slips were glued on the slides with Roti®-Histokitt II (Carl Roth, Karlsruhe, Germany). All stained sections were evaluated under a light-microscope at 60× magnification. For each fish, 10 secondary filaments from the inner section on each slide were analyzed.

Histological analyses were performed according to the methods described by Monteiro et al. (2008) and Mitchell et al. (2012) with slight modifications. Hyperplasia of the primary and secondary filaments was scored at 3 levels of severity, which were defined by the number of layers of epithelial cells as follows: 1) 2–3 was considered as 'minimal', 2) 4–7 was considered as 'moderate' and 3) ≥ 8 was considered as 'severe'. Similar severities of eosinophilic granulocytes were defined based on the number present: ≤ 4 was considered as minimal, 5–8 was considered as moderate, and ≥ 9 was considered as severe. For each severity, the number of cases was noted, and multiplied by the severity factor to quantify the alteration. The severity factor of 'minimal', 'moderate' and 'severe' was defined as 1, 2 and 3, respectively. Finally, the total alteration was determined by the summation of quantified alterations of all severities.